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Original Article

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ABSTRACT

Objectives: To evaluate obestatin levels in both obese and normal subjects before and after Ramadan period. Also, the relationships between plasma obestatin levels and anthropometric and metabolic parameters were analysed.

Design: Prospective study

Setting: Ankara Training and Research Hospital, Ankara, Turkey

Subjects: Thirty obese and 35 normal weight premenopausal women aged between 21 and 42 years were recruited for the study.

Intervention: Blood samples were received from all of the subjects two days before and two days after Ramadan and anthropometric measurements were made.

Main outcome measures: Obestatin was measured by an enzyme immunoassay. Insulin resistance was calculated by homeostasis model assessment of insulin resistance (HOMA-IR).

Results: Obestatin levels were significantly lower in obese individuals compared with normal controls [0.65 (0.72) vs 1.10 (1.22) ng/ml; p <0.01]. While obestatin [1.45 (1.22) vs 0.65 (0.72) ng/ml; p = 0.002] and HDL-C levels were higher after Ramadan in obese group; BMI, HOMA-IR and LDL-C levels were found to be lower. After Ramadan, BMI, HOMA-IR, fasting blood glucose and fasting insulin were negatively correlated with obestatin level.

Conclusions: The changes in pattern of sleep and fasting, and eating two times in a day during Ramadan may lead to increased obestatin in obese subjects and this result may positively affect obesity, glucose and lipid metabolism. Thus, this peptide may be a therapeutic helper in pathological conditions such as insulin resistance and diabetes.

KEY WORDS: fasting, obesity, obestatin, ramadan

INTRODUCTION

Today, the prevalence of obesity is continuously increasing. Thus, the discovery of the molecular mechanisms regulating food intake and body weight also increases. Obestatin was found by Zhang *et al* in 2005[1]. It is produced in the gut and encoded by the ghrelin gene, with 23 amino acid peptide, a hormone. The term obestatin is a contraction of the words “obese” and “statin” denoting suppression[2]. Pathophysiological significance in humans is still unknown.

A matter of debate is the biological role of obestatin. First, it has been proposed as an anorectic hormone and has the opposite effect of ghrelin, but subsequent studies have not supported this view[2]. Obestatin levels are reported to be higher in women than men, does not correlate with age[3], and is non-significantly higher in smokers[4]. It has been suggested that plasma obestatin levels are lower in individuals with obesity[5,6], type 2 diabetes and impaired glucose tolereance (IGT) than individuals with normal weight[7].

The month of Ramadan is holy in Islam and Ramadan fasting is one of the five pillars of Islam. Fasting during Ramadan is obligatory for all healthy Muslims. During Ramadan, Muslims

abstain from food and drink from dawn until sunset. Traditionally the practise is to eat 2 meals, 1 before dawn, suhore, and 1 just after sunset, iftar[8]. Eating and drinking is permitted only at night (between iftar and suhore). A large quantity is eaten in one evening meal instead of in several meals during the day in Ramadan. Ramadan teaches Muslims self-restraint and reminds them of the feelings of the impoverished. According to previous studies, a nutrient given to the body in unusual time may lead to different metabolic effects[8-10]. Sahur which is called the morning breakfast is eaten between 03:00 and 05:00 in the Ramadan month. Also, Ramadan fasting is an opportunity to investigate the effect of reduced frequency of meals (at least two meals a day instead of three meals a day) on body metabolism.

Our hypothesis is that, at the time of Ramadan, and the changes in the number of meals and sleep times of the day, the changes in social habits, including obestatin changes of rhythmic oscillation pattern, the amount of hormone can cause many changes in the body. If any, these changes may differ between normal weight and obese people.

Very little is known about obestatin's physiological role in humans. Thus, to examine the relationship between obestatin with biochemical, anthropometric parameters, and energy metabolism in the body may be interesting. Plasma levels of obestatin in humans, affecting factors, and its interaction with energy metabolism is still limited and requires further investigation. Every information to be obtained about obestatin may be important for this hormone to take place and applicability in the treatment of obesity in the future.

To our knowledge, this is the first study done in relation to levels of obestatin on Ramadan. We know that lipid profile is affected by dietary habits, oxygen saturation, % of daily dietary fat, carbohydrates and exercise. There is limited and contradictory information in relation to lipid levels in Ramadan. In this study, lipid levels were also specially evaluated and its relationships with obestatin were examined.

SUBJECTS AND METHODS

This study was conducted in June-July period of 2015 during the month of Ramadan. Thirty fasting obese subjects (mean age 35, range: 21-42 years, BMI = 34.56 ± 4 kg/m^2) and 35 fasting normal healthy premenopausal women (mean age 34, range: 20 - 40 years, BMI = 23.6 ± 1.4 kg/m^2) (control group) were recruited to the study. Male patients; diabetes mellitus, hypertension, hyperlipidemia, heart failure, liver cirrhosis, cancer, chronic kidney disease, adrenal and thyroid gland disease, prolactinoma (hyperprolactinemia), and acromegaly patients; smokers; pregnant and lactating ones; carbohydrate and lipid metabolism, and the studied parameters will affect any drug users were excluded from the study. Six women in the obese group and 7 women in the control group were excluded from the study because of menstruation during this period. Obese and control groups who were normally menstruating were tested in the follicular phase of their menstrual cycle (days 5-10 from menses).

The local ethics committee approved the study and subjects gave written informed consent before study participation.

Blood samples were received from the study participants two days before Ramadan and during the last 2 days of Ramadan as two times and related measurements were made. Obestatin levels and anthropometric and biochemical parameters were evaluated. Blood sample analyses and anthropometric measurements were done in the same center (Ankara Training and Research Hospital) and by the same staff. Anthropometric measurements were performed at the same time of blood sampling and included the body weight and body mass index (BMI). Body weights of subjects were measured using the TANITA-Feed Body Composition Analyzer (Model TBF-300, Tokyo, Japan)) (BW, % fat and BMI determined) in the morning. Blood samples were taken after a night fast at 08.00 hours before Ramadan and 12 hours after last meal (17.00 hours) on the 28-30th day of Ramadan in all subjects. Plasma was collected and kept at -80 ˚C until assayed. The concentration of plasma obestatin was determined with commercial radioimmunoassay (RIA) kits (Phoenix Pharmaceuticals Inc, Belmont, CA, USA) using ^{125}I-labelled obestatin as a tracer. The intra-assay coefficient of obestatin was 5%, and the interassay coefficient 9%. The sensitivity of the assay was 50 ng/l. Fasting plasma glucose levels were measured by standard enzymatic methods (Roche Diagnostic GmbH, Manheim, Germany). Serum insulin levels were measured by immunoradiometric assay (sandwich-type assay) using an insulin IRMA kit (Immunotech, Prague, Czech Republic). Intra-assay and inter-assay coefficients of variation were 4.3% and 3.4%, respectively; and the minimum detectable insulin concentration was 0.5 µIU/ml. Insulin resistance was determined using the homeostasis model assessment-insulin resistance (HOMA-IR) index, calculated as:

HOMA-IR = [serum glucose level (mg/dl) × insulin (µIU/ml)] / 405

Serum concentrations of total cholesterol (TC), HDL cholesterol (HDL-C), and triglycerides (TG) were measured by enzymatic calorimetric methods using commercially available kits (Roche Diagnostic GmbH). LDL cholesterol (LDL-C) levels were calculated using the Friedewald equation. Body mass index (BMI) was calculated as weight (kg) divided by height (m^2). Normal weight was defined as a BMI of 19 - 24.9 kg/m^2 and obesity was defined as a BMI >30 kg/m^2.

Statistical Analysis

Data analysis was performed by using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, United States). Whether the continuous variables were normally distributed or not were determined by using Shapiro Wilk test. Data were shown as mean ± standard deviation or median (IQR), where applicable. The mean differences between groups were compared by Student's t test, otherwise, Mann Whitney U test was applied for the comparison of the median values. Intra-group comparisons were evaluated by paired t test or Wilcoxon Sign Rank test, where appropriate. Degrees of association between continuous variables were evaluated by Spearman's Rank Correlation test. Multiple linear regression analyses were used to determine

the independent predictors which mostly affected differences in obestatin levels. Coefficient of regression and 95% confidence intervals (CIs) for each independent variable were also calculated. Logarithmic transformations were applied for obestatin in linear regression analyses because of the not normally distributed. A p-value less than 0.05 was considered statistically significant. The Bonferroni Correction was applied for intra-group comparisons controlling Type I error.

RESULTS

The clinical and biochemical characteristics of the study groups are demonstrated in Table 1. While a significant difference in age is not present between obese and normal weighted groups (p = 0.416), there was a significant difference in BMI between the two groups ((34.6 ± 4 vs 23.6 ± 1.4 kg/m^2; p <0.001) as expected.

Obestatin levels were significantly lower in obese individuals compared with normal controls [0.65 (0.72) vs 1.10 (1.22) ng/ml; p<0.01]. In the obese group, HOMA-IR [(3 (1.5) vs 1.5 (1.5); p <0.001], fasting blood glucose [96 (12) vs 86.5 (11.5) mg/dl; p <0.01], fasting insulin [13.5 (6.4) vs 9.4 (9.3) µIU/ml; p <0.01], TG [169 (67.7) vs 101.5 (54.2) mg/dl; p =0.001), TC (196.8 ± 27.9 vs 173.6 ± 22.1 mg/dl; p = 0.001) and LDL-C (121 ± 23.6 vs 103.2 ± 21.7 mg/dl; p0.01) values were found to be significantly higher and HDL-C (44.5 ± 7.8 vs 49.9 ± 8.3 mg/dl; p <0.05) levels was found to be significantly lower before Ramadan (Table 1). When the intra-group values were compared before and after Ramadan; while obestatin [1.45 (1.22) vs 0.65 (0.72) ng/ml; p =0.002] and HDL-C (49.5 ± 8.5 vs 44.5 ± 7.8 mg/dl; p <0.001*)* levels were significantly higher after Ramadan in obese group; BMI (33.1 ± 3.5 vs 34.6 ± 4 kg/m^2; p <0.05), HOMA-IR [2.6 (2) vs 3 (1.5); p <0.05), fasting blood glucose [90.5 (10.2) vs 96 (12) mg/dl; p <0.05], fasting insulin [11.7 (7.5) vs 13.5 (6.4) µIU/ml; p <0.05], TG [159.5 (66.2) vs 169 (67.7) mg/dl; p <0.001] and LDL-C (113.6 ± 21.5 vs 121 ± 23.6 mg/dl; p <0.05) levels were found to be significantly lower.

When the control group's values were compared before and after Ramadan; while obestatin [1.43 (1.18) vs 1.10 (1.22) ng/ml; p = 0.061] levels tended to be higher at the end of Ramadan, the difference was not statistically significant. At the same time, HDL-C (55.1 ± 7.7 vs 49.9 ± 8.3 mg/dl; p <0.001) levels were significantly higher at the end of Ramadan, BMI (23.1±1.3 vs 23.6 ± 1.4 kg/m^2; p <0.01), LDL-C (91.1 ± 25 vs 103.2±21.7 mg/dl; p <0.001) and TG [95 (37) vs 101.5 (54.2) mg/dl; p <0.05] levels were found to be significantly lower. There were no significant differences in HOMA-IR, FBG, fasting insulin, and TC levels.

The results of Spearman's correlation analysis between plasma obestatin levels and selected variables are demonstrated in Table 2.

In the analysis before Ramadan for all subjects, plasma obestatin level was negatively correlated with BMI (r = -0.27, p <0.05), HOMA-IR (r = -0.29, p <0.05), TC (r = -0.26, p <0.05), LDL-C (r = -0.26, p <0.05) and FBG (r = -0.24, p = 0.051) levels. Both groups were examined separately before Ramadan, obestatin levels were not associated with any parameter both in

obese and control groups. In the analysis after Ramadan for all subjects, BMI ($r = -0.26$, $p < 0.05$), HOMA-IR ($r = -0.35$, $p < 0.01$) and fasting insulin ($r = -0.37$, $p < 0.01$) levels were negatively correlated with obestatin levels. After Ramadan, when the data were analyzed separately for each group; BMI ($r = -0.57$, $p = 0.001$), HOMA-IR ($r = -0.44$, $p < 0.05$), FBG ($r = -0.52$, $p < 0.01$) and fasting insulin ($r = -0.41$, $p < 0.05$) were negatively correlated with obestatin levels in the obese group. Obestatin levels were not correlated with any parameter in the control group.

DISCUSSION

In this study, plasma obestatin levels were found to be statistically low in obese individuals when compared to normal controls. This study was conducted during Ramadan. Considering Ramadan fasting; obestatin levels, anthropometric and metabolic parameters, both before and at the end of Ramadan in obese and healthy normal weight control subjects were evaluated. Although obestatin and HDL-C levels were statistically increased at the end of Ramadan, LDL-C levels are found to be statistically decreased in the obese group. In the normal group before Ramadan, there was an increase tendency on obestatin levels which did not reach to a statistical significance, whereas at the end of Ramadan, decrease on LDL-C and increase on HDL-C was determined. Thus, an increase on obestatin and HDL-C as well as a decrease on LDL-C during Ramadan, we can consider the month of Ramadan as a protective period against heart and circulation system diseases (atherosclerosis).

Obestatin is a hormone with 23 amino acids, produced in the gut. Obestatin, which is coded by ghrelin gene, was found in 2005[1,2]. Obestatin levels are not affected by age[3], and higher in women[3]. It is reported that obestatin concentrations are lower in individuals with obesity[5,6] and type 2 diabetes mellitus[7]. Trovato *et al*[11] found that obestatin levels decrease along with food intake in healthy individuals.

It is reported that there was no change on TC, TG and VLDL-C, an increase on LDL-C, decrease on HDL-C and glucose as well as decrease on BW and BMI for both sexes during Ramadan[8]. Then, Mansi *et al*[12] found that Ramadan fasting caused non-significant increase in blood sugar and triglyceride levels, increase in HD-C as well as decrease in weight and LDL-C for healthy students in Jordan. Both groups stated that the effect of Ramadan fasting on serum lipid levels can be closely related with biochemical response to fasting or nutritional diet[8,12].

Although Al Hourani *et al*[13] reported a significant decrease in serum TG and insignificant increase in HDL-C during Ramadan, Sarraf Zadegan *et al*[14] reported that TC, TG, LDL-C, HDL-C and fasting blood glucose levels did not change during Ramadan. According to Rehman *et al*[15], blood glucose, cholesterol, triacylglycerol (TAG) and LDL-C levels were significantly decreased and HDL-C increased during Ramadan.

It is reported that these beneficial changes observed during Ramadan are temporary, and if dietary habits of Ramadan are sustained, it continues[15]. There is a marked decrease in the

activity of HMG-CoA-reductase during fasting, resulting in reduced synthesis of cholesterol depicted as low blood cholesterol levels[15].

It is demonstrated that LDL-C significantly decreased at the end of fasting and there was non-significant increase in HDL-C, TG, VLDL-C and blood glucose. Ramadan fasting has a beneficial affect on LDL-C[16]. In the study of Ali RU *et al*, although there is an increase in HDL-C during Ramadan, there was no change in BMI, TC and LDL-C[17]. In our study, LDL-C decreased and HDL-C increased for both groups at the end of Ramadan.

Coronary artery disease is the main reason for death in the world. This disease and its relation with serum LDL-C and HDL-C are well determined. High levels of LDL-C and low levels of HDL-C are seen along with the rapid development of atherosclerosis. Compared to before Ramadan, LDL-C decreased and HDL-C increased in the middle and at the end of Ramadan. Ramadan fasting is well tolerated and an effective lipid-lowering agent[18].

There are studies which show correlation between obestatin and BMI[3,5-7]. Our results were consistent with a negative relationship between obestatin and BMI as with most studies. In two studies[19,20], it was reported that obestatin levels are higher for obese cases. In a study with healthy normal women, obestatin and BMI, insulin and ghrelin / obestatin rate does not have a significant relation[19]. In another study, a significant relation was observed between fasting obestatin level and measurements of body composition in normal weight women and all of the cohort, however significant relation was not available for obese women. It was reported that possible reason for that was suppressed obestatin secretion as a result of obesity on obese women[5].

As previously reported, obestatin is not endogenously ligand for GPR39[21,22]. Nakahara *et al*[23] suggested that obestatin is a nutritional marker, which reflects body fat and insulin resistance. Haider *et al*[24] demonstrated an increase on obestatin concentrations after weight loss in obese patients, like ghrelin. Observations of Green *et al* support the role of obestatin for regulation of metabolism by means of changes on appetite, but it shows no direct effects on glucose homeostasis or insulin secretion[25]. A study of Ren *et al*, suggests that obestatin regulates ß cell survival and insulin secretion. Therefore, ghrelin-obestatin system is referred as a promising target for treatment of diabetes and obesity[26].

Obestatin is still a debated peptide due to its most conflicting receptor(s). It was shown that obestatin is bound to pancreatic ß cells and glucagon like peptide-1 receptor (GLP-1R) at adipocytes. GLP-1R has recently emerged among the possible receptor candidates. It was shown that obestatin forms antiapoptotic effects on in-vitro and in-vivo ß cells and islets as well as improves the insulin sensitivity. Due to the strong similarity between obestatin and glucagon like peptide-1 (GLP-1) on ß-cell survival effects and activated signaling pathway, it was hypothesized that obestatin would interact with GLP-1R. Also, it was found that obestatin was bound to GLP-1R and upregulated the GLP-1RmRNA. Obestatin binding was displaced by GLP-1R agonist Ex-4[11,27]. The role of obestatin on insulin secretion is still controversial, as

both stimulatory and inhibitory effects were reported[27]. Granata *et al*[28] showed that obestatin increases insulin secretion in both absence and presence of glucose[10]. We also found apparent higher insulin levels in obese group both before and after Ramadan.

Obestatin behave as anti-diabetogenic peptides by positively influencing glucose and lipid metabolism[27,28]. It was shown that obestatin exerts effects, by supressing food intake, slowing gastric emptying and jejunal motility, and reducing body weight gain in rodents[27,29]. Obestatin also regulates lipid metabolism by inhibiting lipolysis in rats[27].

This study has some restrictions. Although blood control time is appropriate for Ramadan fasting, it is not appropriate for physiological circadian during the last few days of Ramadan (08.00 am vs. 05.00 pm.). Therefore, it is important to note this situation during comparisons. Additionally, lipid levels can be affected by physical activities. It is known that physical activities reduce during month Ramadan, however we did not measure the physical activity levels. The third restriction involves the measurements of ghrelin, acilghrelin and des acil ghrelin, which I wish was possible. We are now scheduling such a study.

CONCLUSION

Obestatin can be a beneficial marker for nutritional status, which reflects adiposity and insulin resistance in the short run. In order to think it as an endocrine marker, which can reflect changes on acute and chronic nutritional status, further studies, which shall describe the roles of obestatin and its receptor as well as showing it as a potential treatment against obesity, are needed.

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Authors Contribution: CC designed and wrote manuscript; SG collected data; YA edited manuscript

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Table 1: The clinical and biochemical characteristics of women with normal weight and obese before and during Ramadan

	Before Ramadan			During Ramadan					
Variables	**Obese**	**Normal**	**p**	**Obese**	**Normal**	**p***	**p****	**Variation**	**p*****
n	30	35							
Age (year)	35.5 ± 6.6	34.2 ± 5.2	0.416						
				33.1 ±				-1.5 (17.2)	
BMI (kg/m2)	34.6 ± 4	23.6 ± 1.4	0.01	3.5	23.1 ± 1.3	0.05	0.05	1.0 (10.7)	0.048
								-3.0 (9.4)	
HOMA-IR	3 (1.5)	1.5 (1.5)	0.001[a]	2.6 (2)	1.7 (0.9)	0.05[b]	NS	-0.2 (7.1)	0.053
				90.5				-0.1 ± 17.3	
FBG (mg/dl)	96 (12)	86.5 (11.5)	0.01[a]	(10.2)	90.0 (6)	0.05[b]	NS	-2.4 ± 18.8	0.130
Fasting insulin				11.7				-7.4 ± 18.2	
(µIU/ml)	13.5 (6.4)	9.4 (9.3)	0.01[a]	(7.5)	8.0 (5.4)	0.05[b]	NS	12.1 ± 20.1	0.239
	196.8 ±			196.7 ±	171.2 ±			5.0 ± 5.4	
TC (mg/dl)	27.9	173.6 ± 22.1	0.001	23.6	27.4	NS	NS	5.2 ± 7.4	0.626
	121 ±			113.6 ±				-3.5 (28)	
LDL-C (mg/dl)	23.6	103.2 ± 21.7	0.01	21.5	91.1 ± 25	0.05	0.001	1.0 (30.5)	0.332
				49.5 ±				0.8 (1.6)	
HDL-C (mg/dl)	44.5 ± 7.8	49.9 ± 8.3	0.001	8.5	55.1 ± 7.7	0,001	0.001	0.5 (1.6)	0.882
				159.5				-1.5 ± 2.3	
TG (mg/dl)	169 (67.7)	101.5 (54.2)	0.001[a]	(66.2)	95 (37)	0.001[b]	0.05[b]	-0.5 ± 1.0	0.809
	0.65			1.45				-0.4 (1.6)	
Obestatin (ng/ml)	(0.72)	1.10 (1.22)	0.01[a]	(1.22)	1.43 (1.18)	0.01[b]	0.061[b]	0.0 (0.8)	0.777

Data are expressed as means±SEM, [a]with Mann Whitney U test, [b]with Wilcoxon Sign Rank test.

BMI: body mass index; HOMA-IR: homeostasis model assessment- insulin resistance; FBG: fasting blood glucose; TC: total cholesterol; LDL-C: LDL cholesterol; HDL-C:HDL cholesterol; TG: triglycerides.

p: demonstrates difference between obese and normal subjects before Ramadan; p*: demonstrates difference between obese subjects before and during Ramadan; p**: demonstrates difference between normal subjects before and during Ramadan; p***: demonstrates difference between groups in terms of change before and during Ramadan; $p < 0.05$ was accepted as statistically sifgnificant.

Table 2. Spearman correlation coefficients of obestatin levels with selected variables in obese and normal weighted women before and during Ramadan

Variables	Before Ramadan						During Ramadan					
	All subjects		Obese		Control		All subjects		Obese		Control	
	r	p	r	p	r	p	r	p	r	p	r	p
Age (year)	-0.03	0.77	-0.12	0.5	0.19	0.25	-0.11	0.39	-0.21	0.25	0.06	0.72
BMI (kg/m2)	-0.27	**0.03**	-0.13	0.5	0.05	0.77	-0.26	**0.04**	-0.57	**0.001**	-0.06	0.71
HOMA-IR %	-0.29	**0.02**	-0.34	0.07	0.10	0.55	-0.35	**0.004**	-0.44	**0.015**	-0.26	0.14
FBG (mg/dl)	-0.24	**0.051**	-0.16	0.41	-0.21	0.24	-0.03	0.84	-0.52	**0.004**	0.33	0.06
Fasting insulin (µIU/ml)	-0.19	0.13	-0.26	0.17	-0.05	0.79	-0.37	**0.002**	-0.41	**0.023**	-0.31	0.08
TC (mg/dl)	-0.26	**0.04**	-0.11	0.58	-0.16	0.36	-0.24	**0.055**	-0.12	0.52	-0.30	0.08
LDL-C (mg/dl)	-0.26	**0.04**	-0.08	0.64	-0.14	0.44	-0.24	**0.052**	-0.01	0.96	-0.33	0.06
HDL-C (mg/dl)	-0.07	0,58	-0.16	0.4	-0.22	0.2	0.06	0.64	0.03	0.88	-0.05	0.76
TG (mg/dl)	-0.21	0.10	-0.18	0.34	0.05	0.79	-0.12	0.33	0.003	0.99	-0.06	0.73

BMI: body mass index; HOMA-IR: homeostasis model assessment- insulin resistance; FBG: fasting blood glucose; TC: total cholesterol; LDL-C: LDL cholesterol; HDL-C:HDL cholesterol; TG: triglycerides; $p < 0.05$ statistically significant.